Gene Transfer into Human Lymphocytes by means of retroviral scFv cell targeting vectors

The invention relates to the gene transfer into human lymphocytes, in particular T-lymphocytes using retroviral scFv cell targeting vectors and the use of said vectors for gene therapy, vaccination therapy or diagnostics, in particular for the therapy of T-cell-associated diseases.

The majority of retroviral vectors currently used in gene therapeutic research are derived from the amphotropic murine leukemia virus (MLV). The host cell range of the amphotropic MLV is determined by the surface envelope protein (SU) encoded by the env gene. The protein products of the env gene form the outer envelope of the retroviral vector. The SU proteins interact with i.e. bind to a particular protein (receptor) on the surface of the host cell. The env gene products of the amphotropic MLV allow the gene transfer in a large number of different mammal cells. However, a selective gene transfer into specific cell or tissue types of human or other mammals is not possible with amphotropic MLV vectors since the receptor for the MLV envelope protein on the surface of mammal cells which mediates the entry of amphotropic MLV vectors and the gene transfer may be found on nearly all these cells. Thus, the host cell range of the amphotropic MLV is not specific.

A host cell specificity e.g. is advantageous for the gene therapeutic use, since in a gene therapy outside of the organism (*ex vivo*) (Anderson et al., 1992; Yu et al., 1997) extensive purifications of the cells are avoided. For the therapeutic, diagnostic or vaccination use *in vivo* it is desired that retroviral vectors specifically target the desired host cells prior to the transfer of the therapeutic gene. A restriction of the host cell range of the amphotropic MLV could be achieved by modification of the surface envelope protein. A modification of the surface envelope protein was carried out by fusion with a hormone domain. The cells bearing the hormone receptor were transduced (Kasahara et al., 1995). Furthermore, the surface envelope protein has been modified by fusion with a single chain antibody fragment (*single chain variable fragments*, in the following also referred to as "scFv"). The fragment represented the antigen binding domain of an antibody and is a fusion protein composed of the variable domain Vh and Vl of a monoclonal antibody. The two domains are bound via a glycine and serine oligopeptid [(-(ser-gly4)3-gly-)] enabling the correct folding of the fusion protein (Huston et al, 1991; Whitlow et al., 1991). All modifications carried out heretofore of the MLV surface envelope protein with a scFv show that although the vectors bound to the host target cell no entry into the cell occurred (Russel et al., 1993). Furthermore, it is known that the surface envelope protein of the MLV generally enables no extensive modifications (Cosset et al., 1995). Modifications in which a part of the binding domain of the MLV-SU protein has been replaced often led to an incorrect processing and thus to a defective

transport of the SU protein to the cell surface (Weiss et al., 1993; Morgan et al., 1993; Russel et al., 1993). Thus, the development of cell specific retroviral vectors on the base of MLV having altered surface envelope proteins is only less promising.

Retroviral vectors on the base of Spleen Necrosis Virus SNV are more suitable for a targeted gene transfer into e.g. human cells since the surface envelope protein of SNV enables extensive modifications and is also correctly processed (Martinez and Dornburg 1995; Chu and Dornburg, 1994, 1995; Jiang et al., 1998). For the preparation of such vectors at least two components are required. To the one hand, a so-called expression construct has to be prepared which enables a packaging into and the transfer through a retrovirus. The expression construct comprises a coding DNA fragment of the desired gene product, e.g. a gene for gene therapy or as a vaccine. The expression construct has to comprise a nucleotide sequence referred to as packaging signal psi (ψ) which directs the efficient packaging of the mRNA into retroviral particles. Further, a packaging or helper cell is required which provides the gag, pol and env gene products of SNV without packaging the gag, pol and env genes into a retrovirus. The gag, pol and env genes present in the packaging cell have to be psi-negative. After transfer of the expression construct by transfection of the corresponding plasmide DNA into the packaging cells retroviral particles are delivered into the cell culture supernatant, said particles containing the expression construct and being able to transfer only this gene but not the gag, pol and env genes into the target cell. These vectors are unable to propagate and run only through one replication round. The general process for the preparation of propagation unable retroviral vectors is state of the art (Russel et al., 1993, Cosset et al., 1995; Weiss et al., 1993; Morgan et al., 1993; Martinez and Dornburg, 1995; Chu and Dornburg, 1994, 1995 ; Jiang et al., 1998).

Also the tropism (host cell specificity of the Spleen Necrosis Virus) is determined by the surface envelope protein (SU protein) encoded by the SNV env gene. The SNV surface envelope wild type protein does not permit any selective gene transfer into particular cells or tissues of humans, since the specific recipient protein (receptor) is not present on the surface of human cells (Dornburg, 1995). Therefore, a process has been developed by Dornburg et al., to replace the SNV SU protein for the antigen recognizing domains of antibodies. Said [SNV scFV Env] vectors with four different scFv known heretofore were able to transfer the psi-positive reporter gene, i.e. the bacterial β galactosidase, into selected human target cells (Chu et al., 1994; Chu et al., 1995; Chu and Dornburg, 1997). In detail, there are two scFv expressed against unknown surface antigens on breast and colon carcinoma cells (Chu et al., 1995; Chu and Dornburg, 1997; Jiang et al., 1998), i.e. an scFv directed against the human transferrine receptor and an scFv which recognizes the CD34 surface antigen. A packaging cell line (DSH CXL) has been developed, containing both the psi-negative SNV genes gag, pol and env and the psi-positive reporter-gene (pCXL). Following transfection of the packaging cell with the plasmide DNA of a further expression gene (pTC 53 [expression

vector pTC53 and pTC53zeo Jiang et al., 1998]), in which the entire surface envelope protein has been replaced against a single chain antibody fragment (scFv), retroviral vectors were delivered into the cell supernatant which bore in addition to the surface envelope wild-type protein also the chimeric [scFv-Env] surface protein on their surface. By means of said vectors the reporter gene could be transferred into the scFv-specific target cells. In the process described by Dornburg et al., for the preparation of cell specific retroviral vectors it is true that only already known and cloned scFv may be used.

DE 19752854 A1 describes a method for the preparation of cell type-specific targeting vectors derived from SNV. Up to now, 4 scFv-SNV targeting vectors have been described. They are directed against tumor markers, the transferrine receptor and the CD34 surface antigen (Chu & Dornburg, 1995, 1997, Jiang et al., 1997). Here, the scFv have been derived from monoclonal antibodies (mAb). Furthermore, pseudotype vectors of the type MLV (HIV) for specific transduction of human CD4-positive T cells have been described already (Schnierle & Stitz et al., 1997).

However, no vectors have been described up to now, which are able to transduce human T-cells in a CD4-independent manner.

Thus, an object of the present invention was to provide T-cell specific vectors which are able to transduce T cells in a CD4-independent manner.

The object is solved by cell targeting vectors containing a DNA sequence encoding a single chain antibody fragment (single chain variable fragment, scFv), wherein the single chain antibody fragment has an amino-acid sequence according to any of the figures 1 to 5.

In a preferred embodiment the cell targeting vector further contains a DNA sequence encoding a SNV-env leader according to any of the figures 1 to 5. The cell targeting vectors according to the present invention are T-cell-specific, i.e. the vectors selectively induce human T cells in a CD4 independent manner.

In a further preferred embodiment, the cell targeting vector is derived from SNV (Spleen Necrosis Virus), particularly preferred is the vector pTC53 derived from SNV.

In a further embodiment of the present invention the cell targeting vectors of the invention contain a therapeutic gene. Thus, the invention also relates to the use of the cell targeting vectors of the invention for gene therapy, vaccination therapy or diagnostics.

By having the scFv vectors of the invention the first scFv cell targeting vectors are available which are able to transduce human T cells in a CD4-independent manner with a differently high efficiency.

By means of the vectors of the invention, it is now possible to treat following T-cell associated diseases.

(i) Severe Combined Immunodeficiency (SCID). This is a defect in the adenosine-desaminase gene (ada) or the gene encoding thyrosin kinase JAK-3 (Macchi et al., 1995). As

a therapeutic Gene the intact ada gene is transferred into T cells by means of the vectors of the present invention.

(ii) Acquired Immunodeficiency Syndrome (AIDS) is caused by HIV-1 infection. Therapeutic genes should inhibit the replication or integration of the virus. As therapeutic gene products for intracellular immunization ribozymes, decoy RNA, transdominantly negative mutants of HIV proteins or antibody fragments are suitable (Chang et al., 1994, Ramenzani et al., 1997, Smith et al., 1996, Leavitt et al., 1996, Duan et al., 1995, Levy-Mintz et al., 1996). These therapeutic genes are transferred into the T cells of HIV-1-infected patients by the use according to the invention of the novel cell targeting vectors.

It has been shown that by means of the vectors of the invention (e.g. vectors containing scFv 7A5 shown in Fig. 1; in the following referred to as 7A5 vectors) human macrophages are transduced with a 95% efficiency. Thus, the transfer of therapeutic genes is also possible in HIV-1-infected macrophages by means of said 7A5 vectors.

(iii) T-cell-associated lymphomas.

The (scFv-SNV-Env) targeting vectors of the invention containing a DNA sequence encoding a single chain antibody fragment (single chain variable fragment, scFv), wherein the single chain antibody fragment has an amino-acid sequence (or a fragment) according to any of the figures 1 to 5 selectively enable a transduction of human T-cell lines and partly of primary lymphocytes isolated from blood.

Surprisingly, the vectors of the invention show a selectivity for human T cells which is many times over that for other human cells. The 7-A5-vectors, i.e. the vectors encoding the single chain antibody fragment according to Fig. 1 or a portion thereof, showed a selectivity for human T cells which was increased by a factor of 1000 compared to that for other human cells (c.f. Table 2) and a 4-5 times increased selectivity for T cells compared to B cells.

Table 1 represents 5 scFv (in detail: 7A5, K6, 7B2, 7E4, 6C3) and their vector titers on human T cells (C8166), D17 cells (canine osteosarcoma cell line, permissive for SNV) and HeLa cells (human cervical carcinoma cell line).

Table 2 represents the vector titers of 7A5 vectors. From these data the efficiency and specificity for human T-cells are obvious. By means of said 7A5 vectors T cells which have been made quiescent by gene technologically modified SNV vectors and even human macrophages could be transduced in a very effective manner

Thes following examples illustrate the invention and are not construed to be limiting:

Example 1:

Determination of the vector titers of 5 selected scFv on D17, C8166 and HeLa cells.

For this purpose cell culture supernatants were titered in three serial dilutions (1000 µl, 100 µl and 10 µl) in a total volume of 1000 µl by adding 30 µg/ml polybren on the cells (2 x 10^5 D17 and HeLa, 5 x 10^5 C8166). After a 1,5-2 h incubation period the vector containing supernatant was replaced by fresh medium.

Following 48 h an X-gal staining was used to detect transduced cells (Mikawa et al., 1992), and the blue cells were counted. Tab. 1 shows the vector titers of the 5 selected scFv on D17, C8166 and HeLa cells.

The titration on D17 (canine osteosarcoma cell line, Watanabe et al, 1983) functions as a positive control for the vector production. The titre of > 10^6 i.U./ml shows that all 5 scFv packaging cell clones deliver vector particles into the cell culture supernatant with about the same efficiency.

The titer on C8166 cells vary between 10^3 and 10^6 i.U./ml depending on scFv , while the transduction on HeLa cells revealed no appreciable titer. Said fact indicates a high selectivity for human T cells of all 5 scFv vectors. The 7A5 vectors most efficiently transduce human T cells (Table 1).

Tab. 1: Vector titers of the 5 scFv vectors.

	Titer (i.U./ml)		
ScFv	D17	C8166	HeLa
7A5	$>10^6$	1 x 10^6	$<10^2$
K6	$>10^6$	2,5 x 10^5	$<10^1$
7B2	$>10^6$	2 x 10^4	$<10^1$
7E4	$>10^6$	2 x 10^3	$<10^1$
6C3	$>10^6$	2 x 10^3	$<10^1$

Example 2:

Further characterization of the vectors

For a detailed characterization, further transduction experiments were carried out with the vectors. In Table 2, the results of the 7A5 vectors are represented.

Tab. 2: Transduction of different cell types by means of 7A5 and wild type vectors

	Titer (i.U./ml)								
	D17	HeLa	TE671	HT1080	293T	C8166	Molt4/8	Jurkat	A301	huPBMC
WT	$>10^6$	$<10^1$	$<10^1$	$<10^1$	$<10^1$	$<10^1$	$<10^1$	$<10^1$	$<10^1$	$<10^1$
7A5	$>10^6$	$<10^2$	$<10^1$	$<10^1$	$<10^2$	$1x10^5$	$1x10^6$	$3x10^5$	$1x10^5$	$7{,}5x10^4$

The transductions were carried out as described above. As a control, all cells were transdued with wild type vectors (WT). These are vector particles only containing the SNV Env wild type protein and no scFv. They are delivered from the starting packaging cell line DSH-cx1 (Chu & Dornburg, 1995, Jiang et al., 1998) into the culture supernatant. As expected, said vectors were not able to transduce human cells. Only the D17 cells which were permissive for them could be transduced with high efficiency.

The titration with 7A5 vectors showed an efficient transduction of several human T cell lines (C8166, Molt4-8, Jurkat, A301), while other human cell types (HeLa: cervical carcinoma, TE671: rhabdomyosarcoma, HT1080; fibrosarcoma, 293T; medulla renalis) could not be transduced. These results show that 7A5 vectors have a high selectivity for T cells.

An increased selectivity for T cells was also found for cell targeting vectors containing a DNA sequence encoding a single chain antibody fragment according to Figure 2, 3, 4 or 5.

Example 3:

Transduction of primary T cells

For the transduction of primary T cells, primary human PBMC ("peripheral blood mononuclear cells", the isolation of PBMC from blood by means of sucrose density gradient centrifugation is carried out according to standard methods) were isolated from blood.

After a three days stimulation by means of PHA (phytohemagglutinin) and IL-2 the cell population consisted of 98% T lymphocytes (determined by FACS analysis with an antibody against T cell marker CD3 (state of the art).

The transduction of said cells by means of 7A5 vectors revealed an efficiency of 20% vector positive cells (or approx. 1×10^5 i.U./ml). As a comparison, the transduction experiments were carried out with human B cells. These could be transduced 5 times less (approx. 4%) than T cells.

Further, stimulated human PBMC could be transduced also with K6 and 7B2 vectors (i.e. vectors encoding the single chain antibody fragment according to Fig. 2 or 3 or a portion thereof). However, this occurred with an efficiency approx. 10 times less than with the 7A5 vectors.

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448 ATG GAC TAC TGG GGC CAA GGG ACC ACG GTC ACC GTC TCC TCA GGT GGA TGC GGT TCA GGC 507
150 M   D   Y   W   G   Q   G   T   T   V   T   V   S   S   G   G   C   G   S   G  169

508 GGA GGT GGC TCT GGC GGT GGC GGA TCG GAC ATC GAG CTC ACT CAG TCA CCA GCA ATC ATG 567
170 G   G   G   S   G   G   G   G   S   D   I   E   L   T   Q   S   P   A   I   M  189

568 TCT GCA TCT CCA GGG GAG AAG GTC ACC ATG ACC TGC AGT GCC AGC TCA AGT ATA AGT TAC 627
190 S   A   S   P   G   E   K   V   T   M   T   C   S   A   S   S   S   I   S   Y  209

628 ATG CAC TGG TAC CAG CAG AAG CCA GGC ACC TCC CCC AAA AGA TGG ATT TAT GAC ACA TCC 687
210 M   H   W   Y   Q   Q   K   P   G   T   S   P   K   R   W   I   Y   D   T   S  229

688 AAA CTG GCT TCT GGA GTC CCT GCT CGC TTC AGT GGC AGT GGG TCT GGG ACC TCT TAT TCT 747
230 K   L   A   S   G   V   P   A   R   F   S   G   S   G   S   G   T   S   Y   S  249
```

```
748 CTC CCA ATC AGC AGC ATG GAG GCT GAA GAT GCT GCC ACT TAT TAC TGC CAT CAG CGG AGT  807
250 L   P   I   S   S   M   E   A   E   D   A   A   T   Y   Y   C   H   Q   R   S    269

808 AGT TAC CCA TGG ACG TTC GGT GGA GGG ACC AAG CTG GAA ATA AAA CGG GCG GCC GCA TCG  867
270 S   Y   P   W   T   F   G   G   G   T   K   L   E   I   K   R   A   A   A   S    289

868 GGC TCC GGG GGC GGT GGT TCT GGT GGT GGT TCT GGT GGT GGT GGT TCT GGT GGT GGT GGC  927
290 G   S   G   G   G   G   S   G   G   G   S   G   G   G   G   S   G   G   G   G    309
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<210> 3
<211> 990
<212> DNA
<213> Artificial sequence

<220>
<223> Description of the artificial sequence: scFv encoding sequence

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<400> 3
      ATG GAC TGT CTC ACC AAC CTC CGA TCC GCT GAG GGT AAA GTT GAC CAG GCG AGC AAA ATC  60
       M   D   C   L   T   N   L   R   S   A   E   G   K   V   D   Q   A   S   K   I   20

 61 CTA ATT CTC CTT GTG GCT TGG TGG GGG TTT GGG ACC ACT GCC GAA GTT TCG ACT GCC CGA  120
 21  L   I   L   L   V   A   W   W   G   F   G   T   T   A   E   V   S   T   A   R   40

121 GCG GCC CAG CCG GCC ATG GCC CAG GTG CAG CTG CAG CAG TCT GGG ACT GAA CTG GCA ACA  180
 41  A   A   Q   P   A   M   A   Q   V   Q   L   Q   Q   S   G   T   E   L   A   T   60

181 CCT GGG GCC TCA GTG AGG ATG TCC TGC AAG GCT TCT GGC TAC GCC TTT ACT ACC TAC TGG  240
 61  P   G   A   S   V   R   M   S   C   K   A   S   G   Y   A   F   T   T   Y   W   80

241 ATG CAC TGG GTA AAA CAG AGG CCT GGA CAG GGT CTG GAA TGG ATT GGA TAC ATT AAT CCT  300
 81  M   H   W   V   K   Q   R   P   G   Q   G   L   E   W   I   G   Y   I   N   P   100

301 ACC ACT GAT TAT ACT GAC TAC AAT CTG AAG TTC AAG GAC AAG GCC ACA TTG ACT GCA GAC  360
101  T   T   D   Y   T   D   Y   N   L   K   F   K   D   K   A   T   L   T   A   D   120

361 AAA TCC TCC AGT ACA GCC TAC ATG CAA CTG AGC AGC CTG ACA TCT GAG GAC TCT GCA GTC  420
121  K   S   S   S   T   A   Y   M   Q   L   S   S   L   T   S   E   D   S   A   V   140

421 TAT TAC TGT GCA AGA TCG GGG TGG TCC TAT GCT ATG GAC TAC TGG GGG CAA GGG ACC ACG  480
141  Y   Y   C   A   R   S   G   W   S   Y   A   M   D   Y   W   G   Q   G   T   T   160

481 GTC ACC ATC TCC TCA GGT GGA GGC GGT TCA GGC GGA GGT GGC TCT GGC GGT GGC GGA TCG  540
161  V   T   I   S   S   G   G   G   G   S   G   G   G   G   S   G   G   G   G   S   180

541 GAC ATC GAG CTC ACT CAG TCT CCA GCA ATC ATG TCT GCA TCT CCA GGG GAG AAG GTC ACC  600
181  D   I   E   L   T   Q   S   P   A   I   M   S   A   S   P   G   E   K   V   T   200

601 ATA ACC TGC AGT GCC AGC TCA AGT GTA AGT TAC ATG CAC TGG TTC CAG CAG AAG CCA GGC  660
201  I   T   C   S   A   S   S   S   V   S   Y   M   H   W   F   Q   Q   K   P   G   220

661 ACT TCT CCC AAA CTC TGG ATT TAT AGC ACA TCC AAC CTG GCT TCT GGA GTC CCT GCT CGC  720
221  T   S   P   K   L   W   I   Y   S   T   S   N   L   A   S   G   V   P   A   R   240

721 TTC AGT GGC AGT GGA TCT GGG ACC TCT TAC TCT CTC ACA ATC AGC CGA ATG GAG GCT GAA  780
241  F   S   G   S   G   S   G   T   S   Y   S   L   T   I   S   R   M   E   A   E   260

781 GAT GCT GCC ACT TAT TAC TGC CAG CAA AGG AGT AGT TAC CCA TTC ACG TTC GGC TCG GGC  840
261  D   A   A   T   Y   Y   C   Q   Q   R   S   S   Y   P   F   T   F   G   S   G   280

841 ACG AAG CTG GAA ATC AAA CGG GCG GCC GCA TCG GGC TCC GGG GGC GGT GGT TCT GGT GGT  900
281  T   K   L   E   I   K   R   A   A   A   S   G   S   G   G   G   G   S   G   G   300

901 GGT GGT TCT GGT GGT GGT GGT TCT CGT GGT GGT GGT TCT GGC GCC AGC CCA GTC CAG TTT  960
301  G   G   S   G   G   G   G   S   G   G   G   G   S   G   A   S   P   V   Q   F   320

961 ATC CCC CTG CTT GTG GGT CTA GGG ATT TCA                                        990
321  I   P   L   L   V   G   L   G   I   S                                        330
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<210> 4
<211> 946
<212> DNA
<213> Artificial sequence

<220>
<223> Description of the artificial sequence: scFv encoding sequence

<400> 4

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1 ATG GAC TGT CTC ACC AAC CTC CGA TCC GCT GAG GGT AAA GTT GAC CAG GCG AGC AAA ATC   60
1 M  D  C  L  T  N  L  R  S  A  E  G  K  V  D  Q  A  S  K  I                      20

61 CTA ATT CTC CTT GTG GCT TGG TGG GGG TTT GGG ACC ACT GCC GAA GTT TCG ACT GCC CGA  120
21 L  I  L  L  V  A  W  W  G  F  G  T  T  A  E  V  S  T  A  R                     40

121 GCG GCC CAG CCG GCC ATG GCC GAG GTC AAG CTG CAG CAG TCA GGG GCT GAG CTG GTG AGG  180
41 A  A  Q  P  A  M  A  E  V  K  L  Q  Q  S  G  A  E  L  V  R                     60

181 CCT GGA GCT TCA GTG AAG CTG TCC TGC AAG ACT TCT GGC TTC TCC TTC ACC AGC TAC TGG  240
61 P  G  A  S  V  K  L  S  C  K  T  S  G  F  S  F  T  S  Y  W                     80

241 ATG AAC TGG GTG AAG CTG AGG CCT GGA CAA GGC CTT GAG TGG ATT GGC ATG ATT GAT CCT  300
81 M  N  W  V  K  L  R  P  G  Q  G  L  E  W  I  G  M  I  H  P                     100

301 TCC GAT AGT GAA ACT AGT TTA ACT CAG AGG TTC AAG GAC AAG GCC ACA CTG ACT GTA GAC  360
101 S  D  S  E  T  S  L  T  Q  R  F  K  D  K  A  T  L  T  V  D                    120

361 AAA TCC TCC AGC ACA GCC TAC ATG CAA CTC AGC AGC CCG ACA TCT GAG GAC TCT GCG GTC  420
121 K  S  S  S  T  A  Y  M  Q  L  S  S  P  T  S  E  D  S  A  V                    140

421 TAT TAC TGT GCA AGA TCT CTT TAT GCT AAC TAC CCC TCC TGG TTT ACT TAC TGG GGC CAA  480
141 Y  Y  C  A  R  S  L  Y  A  N  Y  P  S  W  F  T  Y  W  G  Q                    160

481 GGC ACC ACG GTC ACC GTC TCC TCA GGT GGA GGC GGT TCA GGC GGA GGT GGC TCT GGC GGT  540
161 G  T  T  V  T  V  S  S  G  G  G  G  S  G  G  G  S  G  G  G                    180

541 GGC GGA TCG GAC ATC GAG CTC ACT CAG TCT CCA ACC ACC ATG GCT GCA TCT CCC GGG GAG  600
181 G  G  S  D  I  E  L  T  Q  S  P  T  T  M  A  A  S  P  G  E                    200

601 AAG ATC ACT ATC ACC TGC AGT GCC AGC TCA AGT ATA AGT TCC AAT TAC TTG CAT TGG TAT  660
201 K  I  T  I  T  C  S  A  S  S  S  I  S  S  N  Y  L  H  W  Y                    220

651 CAG CAG AAG CCA GGA TTC TCC CCT AAA CTC TTG ATT TAT AGG ACA TCC AAT CTG GCT TCT  720
221 Q  Q  K  P  G  F  S  P  K  L  L  I  Y  R  T  S  N  L  A  S                    240

721 GGA GTC CCA GCT CGC TTC AGT GGC AGT GGG TCT GGG ACC TCT TAC TCT CTC ACA ATT GGC  780
241 G  V  P  A  R  F  S  G  S  G  S  G  T  S  Y  S  L  T  I  G                    260

781 ACC ATG GAG GCT GAA GAT GTT GCC ACT TAC TAC TGC CAG CAG GGT AGT AGT ATA CCG TAC  840
261 T  M  E  A  E  D  V  A  T  Y  Y  C  Q  Q  G  S  S  I  P  Y                    280

841 ACG TTC GGA GGG GGG ACC AAG CTG GAA ATA AAA CGG GCG GCC GCA TCG GGC TCC GGG GGC  900
281 T  F  G  G  G  T  K  L  E  I  K  R  A  A  A  S  G  S  G  G                    300

901 GGT GGT TCT GGT GGT GGT GGT TCT GGT GGT GGT GGT TCT GGT GGT G                    946
301 G  G  S  G  G  G  G  S  G  G  G  G  S  G  G                                    315
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<210> 5
<211> 906
<212> DNA
<213> Artificial sequence

<220>
<223> Description of the artificial sequence: scFv encoding sequence

<400> 5

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      ATG GAC TGT CTC ACC AAC CTC CGA TCC GCT GAG GGT AAA GTT GAC CAG GCG AGC AAA ATC   60
       M   D   C   L   T   N   L   R   S   A   E   G   K   V   D   Q   A   S   K   I    20

   61 CTA ATT CTC CTT GTG GCT TGG TGG GGG TTT GGG ACC ACT GCC GAA GTT TCG ACT GCC CGA  120
   21  L   I   L   L   V   A   W   W   G   F   G   T   T   A   E   V   S   T   A   R   40

  121 GCG GCC CAG CCG GCC ATG GCC CAG GTA CAG CTG CAG CAG TCA GGA GCA GAA ATG AAA AAG  180
   41  A   A   Q   P   A   M   A   Q   V   Q   L   Q   Q   S   G   A   E   M   K   K   60

  181 CCC GGG GAG TCT CTG AAA ATC TCC TGT AAG GGT TTT GGA TAC GAC TTT AGC ACC TAC TGG  240
   61  P   G   E   S   L   K   I   S   C   K   G   F   G   Y   D   F   S   T   Y   W   80

  241 ATC GCC TGG GTG CGC CAG ATG CCC GGG AAA GGC CTG GAG TAC ATG GGG CTC ATC TAT CCT  300
   81  I   A   W   V   R   Q   M   P   G   K   G   L   E   Y   M   G   L   I   Y   P  100

  301 GGT GAC TCT GAC ACC AAA TAC AGC CCG TCC TTC CAA GGC CAG GTC ACC ATC TCA GCC GAC  360
  101  G   D   S   D   T   K   Y   S   P   S   F   Q   G   Q   V   T   I   S   A   D  120

  361 AAG TCC ATC AGC ACC GCC TAC CTG CAG TGG AGC AGC CTG AAG GCC TCG GAC ACC GCC ATG  420
  121  K   S   I   S   T   A   Y   L   Q   W   S   S   L   K   A   S   D   T   A   M  140

  421 TAT TAC TGT GCG AGA GTG TCT GGA TAT TGT AGT AGT ACC AGC TGC TAT GAC TAC TAC TAC  480
  141  Y   Y   C   A   R   V   S   G   Y   C   S   S   T   S   C   Y   D   Y   Y   Y  160

  481 TAC TAC ATG GAC GTC TGG GGC CGG GGA ACC CTG GTC ACC GTC TCG AGA GGT GGA GGC GGT  540
  161  Y   Y   M   D   V   W   G   R   G   T   L   V   T   V   S   R   G   G   G   G  180

  541 TCA GGC GGA GGT GGC TCT GGC GGT GGC GGA TCG GAC ATC GTG ATG ACC CAG TCT CCT TCC  600
  181  S   G   G   G   G   S   G   G   G   G   S   D   I   V   M   T   Q   S   P   S  200

  601 ACC CTG TCT GCA TCT GTA GGA GAC AGA GTC ACC ATG ACT TGC CGG GCC AGT CAG AAC ATT  660
  201  T   L   S   A   S   V   G   D   R   V   T   M   T   C   R   A   S   Q   N   I  220

  661 AAT ATC TGG TTG GCC TGG TAT CAG CAG AAA CCA GGG AAA GCC CCT AAG CTC CTG ATC TAT  720
  221  N   I   W   L   A   W   Y   Q   Q   K   P   G   K   A   P   K   L   L   I   Y  240

  721 AAG GCG TCC ACT TTA GAG AGT GGG GTC CCG TCA AGG TTC AGC GGC AGT GGA TCT GGG ACA  780
  241  K   A   S   T   L   E   S   G   V   P   S   R   F   S   G   S   G   S   G   T  260

  781 GAA TTC ACT CTC ACC ATC AGC GGC CTG CAG CCT GAT GAT TTT GCA AGT TAT TAC TGT CAA  840
  261  E   F   T   L   T   I   S   G   L   Q   P   D   D   F   A   S   Y   Y   C   Q  280

  841 CGG TAT GAT AGT GAC TGG TCG TTC GGC CAA GGG ACC AAG CTG GAG ATC AAA CGT GCG GCC  900
  281  R   Y   D   S   D   W   S   F   G   Q   G   T   K   L   E   I   K   R   A   A  300

  901 GCA TCG                                                                        906
  301  A   S
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<210> 6
<211> 329
<212> PRT
<213> Artificial sequence

<220>
<223> Description of the artificial sequence: scFv, encoded by SEQ. ID. NO. 1

<400> 6

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Met Asp Cys Leu Thr Asn Leu Arg Ser Ala Glu Gly Lys Val Asp Gln
  1               5                  10                  15

Ala Ser Lys Ile Leu Ile Leu Leu Val Ala Trp Trp Gly Phe Gly Thr
             20                  25                  30
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Thr Ala Glu Val Ser Thr Ala Arg Ala Ala Gln Pro Ala Met Ala Glu
35 40 45

Val Lys Leu Gln Gln Ser Gly Ala Glu Leu Val Arg Pro Gly Val Ser
50 55 60

Val Lys Ile Ser Cys Lys Gly Ser Gly Tyr Thr Phe Thr Asp Tyr Gly
65 70 75 80

Met Ser Trp Val Lys Gln Ser His Ala Lys Ser Leu Glu Trp Ile Gly
85 90 95

Leu Ile Ser Thr Tyr Tyr Gly Asp Pro Ser Tyr Asn Gln Arg Phe Lys
100 105 110

Gly Lys Ala Thr Met Thr Val Asp Lys Ser Ser Asn Thr Ala Tyr Leu
115 120 125

Glu Leu Ala Arg Leu Thr Ser Glu Asp Ser Ala Ile Tyr Tyr Cys Ala
130 135 140

Arg Ser Asp Gly Asn Tyr Gly Tyr Tyr Tyr Ala Leu Asp Tyr Trp Gly
145 150 155 160

Gln Gly Thr Thr Val Thr Val Ser Ser Gly Gly Gly Gly Ser Gly Gly
165 170 175

Gly Gly Ser Gly Gly Gly Gly Ser Asp Ile Glu Leu Thr Gln Ser Pro
180 185 190

Ser Ser Leu Ala Val Ser Leu Gly Gln Arg Ala Thr Ile Ser Cys Arg
195 200 205

Ala Ser Glu Ser Val Asp Ser Tyr Gly Asp Ser Phe Met His Trp Tyr
210 215 220

Gln Gln Lys Pro Gly Gln Pro Pro Lys Leu Leu Ile Tyr Arg Ala Ser
225 230 235 240

Asn Leu Glu Ser Gly Val Pro Ala Arg Phe Ser Gly Ser Gly Ser Glu
245 250 255

Ser Asp Phe Thr Leu Thr Ile Asp Pro Val Glu Glu Asp Asp Ala Ala
260 265 270

Val Tyr Tyr Cys Leu Gln Ser Met Glu Asp Pro Tyr Thr Phe Gly Gly
275 280 285

Gly Thr Lys Leu Glu Ile Lys Arg Ala Ala Ala Ser Gly Ser Gly Gly
290 295 300

Gly Gly Ser Gly Gly Gly Ser Gly Gly Gly Gly Ser Gly Gly Gly Gly
305 310 315 320

Ser Gly Ala Ser Pro Val Gln Phe Ile
325

<210> 7

<211> 309
<212> PRT
<213> Artificial sequence

<220>
<223> Description of the artificial sequence:scFv, encoded by SEQ. ID. NO. 2

<400> 7

Met Asp Cys Leu Thr Asn Leu Arg Ser Ala Glu Gly Lys Val Asp Gln
1 5 10 15

Ala Ser Lys Ile Leu Ile Leu Leu Val Ala Trp Trp Gly Phe Gly Thr
20 25 30

Thr Ala Glu Val Ser Thr Ala Arg Ala Ala Gln Pro Ala Met Ala Glu
35 40 45

Val Lys Leu Gln Glu Ser Gly Thr Glu Leu Val Lys Pro Gly Ala Ser
50 55 60

Val Asn Leu Ser Cys Lys Ala Ser Gly Tyr Thr Phe Thr Ser Tyr Trp
65 70 75 80

Met His Trp Leu Lys Gln Arg Pro Gly Gln Gly Leu Glu Trp Ile Gly
85 90 95

Glu Ile Asp Pro Val Asp Ser Tyr Thr Asn Tyr Asn Gln Asn Phe Lys
100 105 110

Gly Lys Ala Thr Leu Thr Val Asp Lys Ser Ser Thr Thr Val Tyr Met
115 120 125

His Leu Ser Ser Leu Thr Ser Glu Asp Ser Ala Val Tyr Tyr Cys Ala
130 135 140

Arg Lys Gly Tyr Ala Met Asp Tyr Trp Gly Gln Gly Thr Asn Val Thr
145 150 155 160

Val Ser Ser Gly Gly Cys Gly Ser Gly Gly Gly Gly Ser Gly Gly Gly
165 170 175

Gly Ser Asp Ile Glu Leu Thr Gln Ser Pro Ala Ile Met Ser Ala Ser
180 185 190

Pro Gly Glu Lys Val Thr Met Thr Cys Ser Ala Ser Ser Ser Ile Ser
195 200 205

Tyr Met His Trp Tyr Gln Gln Lys Pro Gly Thr Ser Pro Lys Arg Trp
210 215 220

Ile Tyr Asp Thr Ser Lys Leu Ala Ser Gly Val Pro Ala Arg Phe Ser
225 230 235 240

Gly Ser Gly Ser Gly Thr Ser Tyr Ser Leu Pro Ile Ser Ser Met Glu
245 250 255

Ala Glu Asp Ala Ala Thr Tyr Tyr Cys His Gln Arg Ser Ser Tyr Pro
260 265 270

Trp Thr Phe Gly Gly Gly Thr Lys Leu Glu Ile Lys Arg Ala Ala Ala
275 280 285

Ser Gly Ser Gly Gly Gly Gly Ser Gly Gly Gly Ser Gly Gly Gly Gly
290 295 300

Ser Gly Gly Gly Gly
305

<210> 8
<211> 330
<212> PRT
<213> Artificial sequence

<220>
<223> Description of the artificial sequence: scFv, encoded by SEQ. ID. NO. 3

<400> 8
Met Asp Cys Leu Thr Asn Leu Arg Ser Ala Glu Gly Lys Val Asp Gln
1 5 10 15

Ala Ser Lys Ile Leu Ile Leu Leu Val Ala Trp Trp Gly Phe Gly Thr
20 25 30

Thr Ala Glu Val Ser Thr Ala Arg Ala Ala Gln Pro Ala Met Ala Gln
35 40 45

Val Gln Leu Gln Gln Ser Gly Thr Glu Leu Ala Thr Pro Gly Ala Ser
50 55 60

Val Arg Met Ser Cys Lys Ala Ser Gly Tyr Ala Phe Thr Thr Tyr Trp
65 70 75 80

Met His Trp Val Lys Gln Arg Pro Gly Gln Gly Leu Glu Trp Ile Gly
85 90 95

Tyr Ile Asn Pro Thr Thr Asp Tyr Thr Asp Tyr Asn Leu Lys Phe Lys
100 105 110

Asp Lys Ala Thr Leu Thr Ala Asp Lys Ser Ser Ser Thr Ala Tyr Met
115 120 125

Gln Leu Ser Ser Leu Thr Ser Glu Asp Ser Ala Val Tyr Tyr Cys Ala
130 135 140

Arg Ser Gly Trp Ser Tyr Ala Met Asp Tyr Trp Gly Gln Gly Thr Thr
145 150 155 160

Val Thr Ile Ser Ser Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser Gly
165 170 175

Gly Gly Gly Ser Asp Ile Glu Leu Thr Gln Ser Pro Ala Ile Met Ser
180 185 190

Ala Ser Pro Gly Glu Lys Val Thr Ile Thr Cys Ser Ala Ser Ser Ser
195 200 205

Val Ser Tyr Met His Trp Phe Gln Gln Lys Pro Gly Thr Ser Pro Lys
210 215 220

Leu Trp Ile Tyr Ser Thr Ser Asn Leu Ala Ser Gly Val Pro Ala Arg
225 230 235 240

Phe Ser Gly Ser Gly Ser Gly Thr Ser Tyr Ser Leu Thr Ile Ser Arg
245 250 255

Met Glu Ala Glu Asp Ala Ala Thr Tyr Tyr Cys Gln Gln Arg Ser Ser
260 265 270

Tyr Pro Phe Thr Phe Gly Ser Gly Thr Lys Leu Glu Ile Lys Arg Ala
275 280 285

Ala Ala Ser Gly Ser Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser Gly
290 295 300

Gly Gly Gly Ser Gly Gly Gly Gly Ser Gly Ala Ser Pro Val Gln Phe
305 310 315 320

Ile Pro Leu Leu Val Gly Leu Gly Ile Ser
325 330

<210> 9
<211> 315
<212> PRT
<213> Artificial sequence

<220>
<223> Description of the artificial sequence: scFv, encoded by SEQ. ID. NO. 4

<400> 9
Met Asp Cys Leu Thr Asn Leu Arg Ser Ala Glu Gly Lys Val Asp Gln
1 5 10 15

Ala Ser Lys Ile Leu Ile Leu Leu Val Ala Trp Trp Gly Phe Gly Thr
20 25 30

Thr Ala Glu Val Ser Thr Ala Arg Ala Ala Gln Pro Ala Met Ala Glu
35 40 45

Val Lys Leu Gln Gln Ser Gly Ala Glu Leu Val Arg Pro Gly Ala Ser
50 55 60

Val Lys Leu Ser Cys Lys Thr Ser Gly Phe Ser Phe Thr Ser Tyr Trp
65 70 75 80

Met Asn Trp Val Lys Leu Arg Pro Gly Gln Gly Leu Glu Trp Ile Gly
85 90 95

Met Ile His Pro Ser Asp Ser Glu Thr Ser Leu Thr Gln Arg Phe Lys
100 105 110

Asp Lys Ala Thr Leu Thr Val Asp Lys Ser Ser Ser Thr Ala Tyr Met
115 120 125

Gln Leu Ser Ser Pro Thr Ser Glu Asp Ser Ala Val Tyr Tyr Cys Ala
130 135 140

Arg Ser Leu Tyr Ala Asn Tyr Pro Ser Trp Phe Thr Tyr Trp Gly Gln
145 150 155 160

Gly Thr Thr Val Thr Val Ser Ser Gly Gly Gly Gly Ser Gly Gly Gly
165 170 175

Gly Ser Gly Gly Gly Gly Ser Asp Ile Glu Leu Thr Gln Ser Pro Thr
180 185 190

Thr Met Ala Ala Ser Pro Gly Glu Lys Ile Thr Ile Thr Cys Ser Ala
195 200 205

Ser Ser Ser Ile Ser Ser Asn Tyr Leu His Trp Tyr Gln Gln Lys Pro
210 215 220

Gly Phe Ser Pro Lys Leu Leu Ile Tyr Arg Thr Ser Asn Leu Ala Ser
225 230 235 240

Gly Val Pro Ala Arg Phe Ser Gly Ser Gly Ser Gly Thr Ser Tyr Ser
245 250 255

Leu Thr Ile Gly Thr Met Glu Ala Glu Asp Val Ala Thr Tyr Tyr Cys
260 265 270

Gln Gln Gly Ser Ser Ile Pro Tyr Thr Phe Gly Gly Gly Thr Lys Leu
275 280 285

Glu Ile Lys Arg Ala Ala Ala Ser Gly Ser Gly Gly Gly Gly Ser Gly
290 295 300

Gly Gly Gly Ser Gly Gly Gly Gly Ser Gly Gly
305 310 315

<210> 10
<211> 302
<212> PRT
<213> Artificial sequence

<220>
<223> Description of the artificial sequence: scFv, encoded by SEQ. ID. NO. 5

<400> 10
Met Asp Cys Leu Thr Asn Leu Arg Ser Ala Glu Gly Lys Val Asp Gln
1 5 10 15

Ala Ser Lys Ile Leu Ile Leu Leu Val Ala Trp Trp Gly Phe Gly Thr
20 25 30

Thr Ala Glu Val Ser Thr Ala Arg Ala Ala Gln Pro Ala Met Ala Gln
35 40 45

Val Gln Leu Gln Gln Ser Gly Ala Glu Met Lys Lys Pro Gly Glu Ser
50 55 60

Leu Lys Ile Ser Cys Lys Gly Phe Gly Tyr Asp Phe Ser Thr Tyr Trp
65 70 75 80

Ile Ala Trp Val Arg Gln Met Pro Gly Lys Gly Leu Glu Tyr Met Gly
85 90 95

Leu Ile Tyr Pro Gly Asp Ser Asp Thr Lys Tyr Ser Pro Ser Phe Gln
100 105 110

Gly Gln Val Thr Ile Ser Ala Asp Lys Ser Ile Ser Thr Ala Tyr Leu
115 120 125

Gln Trp Ser Ser Leu Lys Ala Ser Asp Thr Ala Met Tyr Tyr Cys Ala
130 135 140

Arg Val Ser Gly Tyr Cys Ser Ser Thr Ser Cys Tyr Asp Tyr Tyr Tyr
145 150 155 160

Tyr Tyr Met Asp Val Trp Gly Arg Gly Thr Leu Val Thr Val Ser Arg
165 170 175

Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser Asp
180 185 190

Ile Val Met Thr Gln Ser Pro Ser Thr Leu Ser Ala Ser Val Gly Asp
195 200 205

Arg Val Thr Met Thr Cys Arg Ala Ser Gln Asn Ile Asn Ile Trp Leu
210 215 220

Ala Trp Tyr Gln Gln Lys Pro Gly Lys Ala Pro Lys Leu Leu Ile Tyr
225 230 235 240

Lys Ala Ser Thr Leu Glu Ser Gly Val Pro Ser Arg Phe Ser Gly Ser
245 250 255

Gly Ser Gly Thr Glu Phe Thr Leu Thr Ile Ser Gly Leu Gln Pro Asp
260 265 270

Asp Phe Ala Ser Tyr Tyr Cys Gln Arg Tyr Asp Ser Asp Trp Ser Phe
275 280 285

Gly Gln Gly Thr Lys Leu Glu Ile Lys Arg Ala Ala Ala Ser
290 295 300